*THE COVALENT STRUCTURE OF AN ENTIRE γG IMMUNOGLOBULIN MOLECULE**

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Abstract.—The complete amino acid sequence of a human γG1 immunoglobulin (Eu) has been determined and the arrangement of all of the disulfide bonds has been established. Comparison of the sequence with that of another myeloma protein (He) suggests that the variable regions of heavy and light chains are homologous and similar in length. The constant portion of the heavy chain contains three homology regions each of which is similar in size and homologous to the constant region of the light chain. Each variable region and each constant homology region contains one intrachain disulfide bond. The half-cystines participating in the interchain bonds are all clustered within a stretch of ten residues at the middle of the heavy chains.

These data support the hypothesis that immunoglobulins evolved by gene duplication after early divergence of V genes, which specified antigen-binding functions, and C genes, which specified other functions of antibody molecules. Each polypeptide chain may therefore be specified by two genes, V and C, which are fused to form a single gene (translocation hypothesis). The internal homologies and symmetry of the molecule suggest that homology regions may have similar three-dimensional structures each consisting of a compact domain which contributes to at least one active site (domain hypothesis). Both hypotheses are in accord with the linear regional differentiation of function in antibody molecules.

Antibodies or immunoglobulins can interact with a wide range of different antigenic determinants and, after specific binding to an antigen, they play a fundamental part in physiological functions of the immune response. The specificity of antigen binding depends ultimately upon amino acid sequences of the variable or V regions of antibody molecules. It is the diversity of these sequences which results in the range of specificities required for a selective immune response. In contrast, other regions of the antibody molecule have relatively constant sequences and are responsible for physiological functions. Like enzymes, these C regions appear to have evolved for a restricted set of interactions. This unusual picture of intramolecular differentiation has emerged from studies of the structure of immunoglobulins from different animal species.[1] To date, only portions of immunoglobulin molecules have been subjected to amino acid sequence determination.

We now report the amino acid sequence of an entire human γG1 immunoglobulin (molecular weight 150,000), the location of all disulfide bonds, the arrangement of light and heavy chains, and the length of the heavy chain V region.

Materials and Methods.—The isolation of the myeloma protein Eu[3] and the preparation of its CNBr fragments[3, 4] have been described. Similar methods were used for the isolation of the γG1 myeloma protein He and for the preparation of its CNBr fragments.

We have previously described the methods used for enzymatic digestion with trypsin, chymotrypsin, and pepsin, gel filtration, ion exchange chromatography, high voltage paper electrophoresis, determination of NH_2-terminal and COOH-terminal residues, amino acid analysis, and determination of amino acid sequences by the dansyl-Edman procedure.[4-6]

The positions of glutamine and asparagine were assigned[9] by determining the electrophoretic mobility of peptides and by amino acid analysis of the peptides after enzymatic hydrolysis. The half-cystinyl residues contributing to each intrachain disulfide bond were determined[10] using the diagonal electrophoresis method.[11]

Results.—The organization of the whole molecule is shown in Figure 1; an unequivocal proof of the arrangement of the two identical light chains and two identical heavy chains has already been given.[4] Each light chain is linked to its neighboring heavy chain by a disulfide bond between corresponding half-

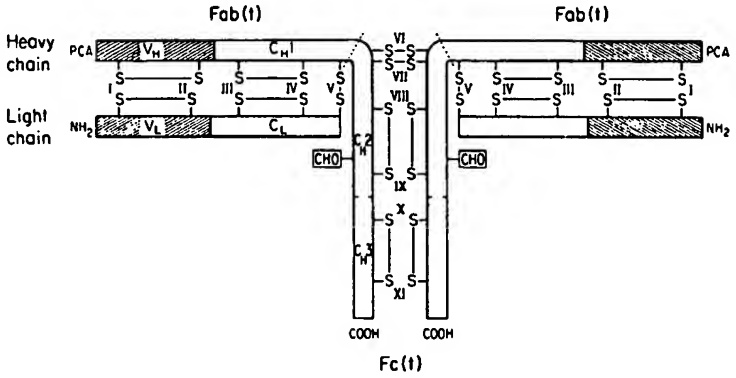

FIG. 1.—Over-all arrangement of chains and disulfide bonds of γG1 immunoglobulin Eu. Half-cystinyl residues are numbered I–XI; numbers I–V designate corresponding residues in light and heavy chains. PCA: pyrollidonecarboxylic acid. CHO: carbohydrate. "Fab(t)" and "Fc(t)" refer to fragments produced by trypsin, which cleaves the heavy chain as indicated by dashed lines above half-cystinyl residues VI. V_H, V_L: variable regions of heavy and light chains, C_L: constant region of light chain. C_H1, C_H2, C_H3: homology regions comprising C_H or constant region of heavy chain.

cystines V. Half-cystines VI and VII form bonds linking the half-molecules via the heavy chains. Trypsin cleaves the molecule at lysyl residue 222 to form two Fab(t) and one Fc(t) fragments.[2, 5]

There are several strikingly linear arrangements in the primary structure. From their amino termini to half-cystines V, the light and heavy chains can be aligned or put in register. The intrachain disulfide bonds are linearly and periodically disposed.[12, 13] In accord with the alignment of light and heavy chains, corresponding intrachain disulfide bonds are in similar positions and the disulfide loops are of approximately the same size.

Previous studies[7] have suggested that V regions of light and heavy chains have similar lengths and begin at the NH_2-termini; this will be confirmed below. The C_L region of the light chain has the same length as V_L, but the C_H region of the heavy chain is about three times as long. C_H may be divided into three

homologous regions of approximately equal length: C_H1, C_H2, and C_H3 (Fig. 1).

We have already reported the amino acid sequence of the first 87[7] and the last 224 residues[8] of the heavy chain as well as the partial sequence of the entire light chain.[6] The complete amino acid sequence of the light chain (214 residues) is shown in Figure 2. Positions of the half-cystinyl residues may be compared with Figure 1 and the methionyl residues may be correlated with previous studies on the CNBr fragments of Eu.[3, 4] The variable region extends through residue 108. In accord with other studies,[1] valine 191 is related to the Inv specificity.[2]

The complete sequence of the heavy chain (446 residues) is presented in Figure 3 which may be compared with Figure 2 for alignment with the light chain sequence. Isolation of a single glycopeptide[8] indicated that the polysaccharide

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1                                                                  10                                                                  20
ASP-ILE-GLN-MET-THR-GLN-SER-PRO-SER-THR-LEU-SER-ALA-SER-VAL-GLY-ASP-ARG-VAL-THR-

                                                                   30                                                                  40
ILE-THR-[CYS]-ARG-ALA-SER-GLN-SER-ILE-ASN-THR-TRP-LEU-ALA-TRP-TYR-GLN-GLN-LYS-PRO-

                                                                   50                                                                  60
GLY-LYS-ALA-PRO-LYS-LEU-LEU-MET-TYR-LYS-ALA-SER-SER-LEU-GLU-SER-GLY-VAL-PRO-SER-

                                                                   70                                                                  80
ARG-PHE-ILE-GLY-SER-GLY-SER-GLY-THR-GLU-PHE-THR-LEU-THR-ILE-SER-SER-LEU-GLN-PRO-

                                                                   90                                                                  100
ASP-ASP-PHE-ALA-THR-TYR-TYR-[CYS]-GLN-GLN-TYR-ASN-SER-ASP-SER-LYS-MET-PHE-GLY-GLN-

                                                                   110                                                                 120
GLY-THR-LYS-VAL-GLU-VAL-LYS-GLY-THR-VAL-ALA-ALA-PRO-SER-VAL-PHE-ILE-PHE-PRO-PRO-

                                                                   130                                                                 140
SER-ASP-GLU-GLN-LEU-LYS-SER-GLY-THR-ALA-SER-VAL-VAL-[CYS]-LEU-LEU-ASN-ASN-PHE-TYR-

                                                                   150                                                                 160
PRO-ARG-GLU-ALA-LYS-VAL-GLN-TRP-LYS-VAL-ASP-ASN-ALA-LEU-GLN-SER-GLY-ASN-SER-GLN-

                                                                   170                                                                 180
GLU-SER-VAL-THR-GLU-GLN-ASP-SER-LYS-ASP-SER-THR-TYR-SER-LEU-SER-SER-THR-LEU-THR-

                                                                   190                                                                 200
LEU-SER-LYS-ALA-ASP-TYR-GLU-LYS-HIS-LYS-VAL-TYR-ALA-[CYS]-GLU-VAL-THR-HIS-GLN-GLY-

                                                                   210                                214
LEU-SER-SER-PRO-VAL-THR-LYS-SER-PHE-ASN-ARG-GLY-GLU-[CYS]
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FIG. 2.—Complete amino acid sequence of the Eu light chain. Half-cystinyl residues are in boxes and methionyl residues are underlined.

portion of the molecule is attached at Asx residue 297.[14] In a previous study[8] we have suggested that glutamyl residue 356 and methionyl residue 358 may be associated with Gm 1 specificities. The sequence of Eu (Gm 4+) between residues 211–252 can be compared with the partial sequence of immunoglobulin Daw[17] (Gm 4−). The presence of arginine in position 214 of Eu and lysine in a comparable position of Daw may be associated with their Gm 4 specificities.[18]

Of particular significance is the determination of the point at which V_H ends and C_H begins. A CNBr fragment comparable to fragment H_4 was isolated from myeloma protein He which has the same Gm specificity as protein Eu. The sequence of the amino terminal portion of the CNBr fragment from He differed from that of the H_4 fragment from Eu.[21]

1 10 20
PCA-VAL-GLN-LEU-VAL-GLN-SER-GLY-ALA-GLU-VAL-LYS-LYS-PRO-GLY-SER-SER-VAL-LYS-VAL-

30 40
SER-[CYS]-LYS-ALA-SER-GLY-GLY-THR-PHE-SER-ARG-SER-ALA-ILE-ILE-TRP-VAL-ARG-GLN-ALA-

50 60
PRO-GLY-GLN-GLY-LEU-GLU-TRP-MET-GLY-GLY-ILE-VAL-PRO-MET-PHE-GLY-PRO-PRO-ASN-TYR-

70 80
ALA-GLN-LYS-PHE-GLN-GLY-ARG-VAL-THR-ILE-THR-ALA-ASP-GLU-SER-THR-ASN-THR-ALA-TYR-

90 100
MET-GLU-LEU-SER-SER-LEU-ARG-SER-GLU-ASP-THR-ALA-PHE-TYR-PHE-[CYS]-ALA-GLY-GLY-TYR-

110 120
GLY-ILE-TYR-SER-PRO-GLU-GLU-TYR-ASN-GLY-GLY-LEU-VAL-THR-VAL-SER-SER-ALA-SER-THR-

130 140
LYS-GLY-PRO-SER-VAL-PHE-PRO-LEU-ALA-PRO-SER-SER-LYS-SER-THR-SER-GLY-GLY-THR-ALA-

150 160
ALA-LEU-GLY-[CYS]-LEU-VAL-LYS-ASP-TYR-PHE-PRO-GLU-PRO-VAL-THR-VAL-SER-TRP-ASN-SER-

170 180
GLY-ALA-LEU-THR-SER-GLY-VAL-HIS-THR-PHE-PRO-ALA-VAL-LEU-GLN-SER-SER-GLY-LEU-TYR-

190 200
SER-LEU-SER-SER-VAL-VAL-THR-VAL-PRO-SER-SER-SER-LEU-GLY-THR-GLN-THR-TYR-ILE-[CYS]-

210 220
ASN-VAL-ASN-HIS-LYS-PRO-SER-ASN-THR-LYS-VAL-ASP-LYS-ARG-VAL-GLU-PRO-LYS-SER-[CYS]-

230 240
ASP-LYS-THR-HIS-THR-[CYS]-PRO-PRO-[CYS]-PRO-ALA-PRO-GLU-LEU-LEU-GLY-GLY-PRO-SER-VAL-

250 260
PHE-LEU-PHE-PRO-PRO-LYS-PRO-LYS-ASP-THR-LEU-MET-ILE-SER-ARG-THR-PRO-GLU-VAL-THR-

270 280
[CYS]-VAL-VAL-VAL-ASP-VAL-SER-HIS-GLU-ASP-PRO-GLN-VAL-LYS-PHE-ASN-TRP-TYR-VAL-ASP-

290 300
GLY-VAL-GLN-VAL-HIS-ASN-ALA-LYS-THR-LYS-PRO-ARG-GLU-GLN-GLN-TYR-ASX-SER-THR-TYR-

310 320
ARG-VAL-VAL-SER-VAL-LEU-THR-VAL-LEU-HIS-GLN-ASN-TRP-LEU-ASP-GLY-LYS-GLU-TYR-LYS-

330 340
[CYS]-LYS-VAL-SER-ASN-LYS-ALA-LEU-PRO-ALA-PRO-ILE-GLU-LYS-THR-ILE-SER-LYS-ALA-LYS-

350 360
GLY-GLN-PRO-ARG-GLU-PRO-GLN-VAL-TYR-THR-LEU-PRO-PRO-SER-ARG-GLU-GLU-MET-THR-LYS-

370 380
ASN-GLN-VAL-SER-LEU-THR-[CYS]-LEU-VAL-LYS-GLY-PHE-TYR-PRO-SER-ASP-ILE-ALA-VAL-GLU-

390 400
TRP-GLU-SER-ASN-ASP-GLY-GLU-PRO-GLU-ASN-TYR-LYS-THR-THR-PRO-PRO-VAL-LEU-ASP-SER-

410 420
ASP-GLY-SER-PHE-PHE-LEU-TYR-SER-LYS-LEU-THR-VAL-ASP-LYS-SER-ARG-TRP-GLN-GLU-GLY-

430 440
ASN-VAL-PHE-SER-[CYS]-SER-VAL-MET-HIS-GLU-ALA-LEU-HIS-ASN-HIS-TYR-THR-GLN-LYS-SER-

446
LEU-SER-LEU-SER-PRO-GLY

FIG. 3.—Complete amino acid sequence of the Eu heavy chain. Half-cystinyl residues are in boxes and methionyl residues are underlined.

A comparison of the sequence of the two fragments from residues 101 to 121 is given in Figure 4. The sequences become identical at residue 115 (Eu numbering). Further studies[21] confirmed that from residue 115 to residue 252 the sequence of the He fragment was identical to that of Eu H_4. In addition, tryptic fingerprints of the Fc fragments from Eu and He were identical. These

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                101                 105                 110                 115                 120
EU   -GLY-ILE-TYR-SER-PRO-GLU-GLU-TYR-ASN-GLY-GLY-LEU-VAL-THR-VAL-SER-SER-ALA-SER-THR-LYS-

HE   -THR-LEU-ALA-PHE-ASX-VAL-TRP-GLY-GLX-GLY-THR-LYS-VAL-ALA-VAL-SER-SER-ALA-SER-THR-LYS-
```

FIG. 4.—Comparison of the amino acid sequence of the Eu heavy chain from residue 101–121 with the corresponding sequence of the heavy chain of myeloma protein He.

data suggest that the transition between V_H and C_H is located in the vicinity of residue 114 (Eu numbering). Studies on a number of additional proteins and a search for V_H region subgroups[7] will be required to locate this point definitively.

Discussion.—The present studies provide proof of the covalent structure and arrangement of chains in γG1 immunoglobulin. The half-molecule of Eu is the largest protein unit (446 + 214 residues) for which a complete amino acid sequence has been determined. In a protein of this size, one cannot neglect the possibility of small errors in sequence assignment; for this reason we are carrying out a number of further checks using various methods of peptide cleavage and fractionation.

One of the most striking features of the immunoglobulin molecule that emerges from the completed sequence is the sharp demarcation of its polypeptide chains into linearly connected regions that are associated with different functions. Variations in the sequences of paired V_H and V_L regions for the function of antigen binding in the selective immune response, and at the same time, conservation of sequence in C_H and C_L regions for other immunological functions appear to require special genetic and evolutionary mechanisms.[22]

The amino acid sequence of Eu provides convincing evidence that the immunoglobulin molecule evolved by successive duplication of precursor genes.[23, 24] Our analysis of a complete heavy chain has revealed an additional homology region (C_H1), the structure of which was not previously known. Earlier comparisons[7, 8] of polynucleotide sequences corresponding to both chains of Eu showed evidence of homology between V_H and V_L and homologies among C_L, C_H2, and C_H3. A complete comparison of the amino acid sequences of C_L, C_H1, C_H2, and C_H3 is given in Figure 5. In a stretch of 100 residues, any two regions are identical in 29 to 34 positions. It is noteworthy that the stretch in the heavy chain from residue 221 to 233 which contains the interchain disulfide bonds[5] has no homologous counterpart in other portions of heavy or light chains.

In the data accumulated so far, little or no homology has been found between V and C regions. This prompts the speculation that V genes and C genes diverged early in the evolution of antibodies to serve two major groups of functions: antigen recognition functions (ARF) and effector functions (EF) such as interaction with cells and complement. The order of emergence of C_L genes or C_H genes from a precursor gene is not apparent from the data. A comparison with sequences of μ, α, δ, or ϵ chains may indicate similarities in their V_H regions[25] and may show whether any of the homology regions are conserved in constant regions of heavy chains of these classes.[26]

Early evolutionary divergence of V and C genes is consistent with the evidence[27, 28] that *each* chain is specified by two genes, V and C, and the hypothesis[7, 22, 29] that V gene episomes are translocated to C genes to form a single VC

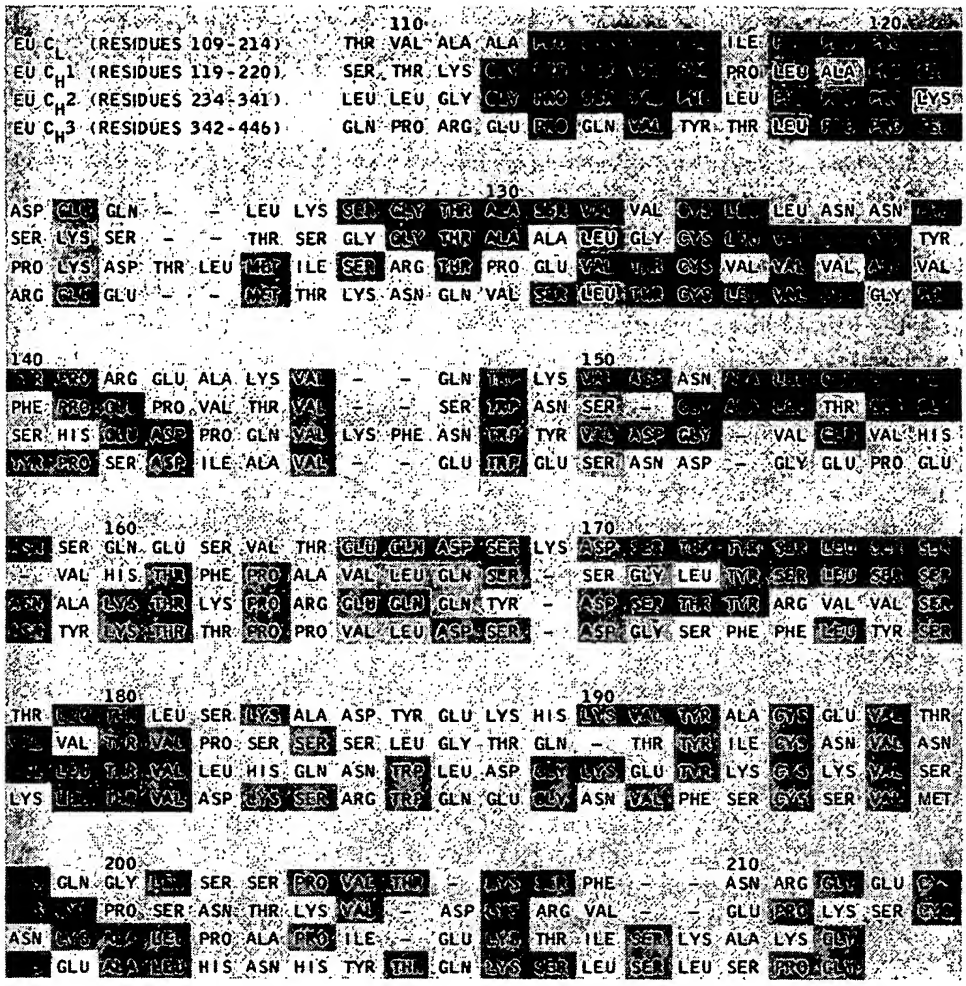

FIG. 5.—Comparison of the amino acid sequence of C_L, C_H1, C_H2, and C_H3. Deletions indicated by dashes have been introduced to maximize the homology. Identical residues are darkly shaded; both dark and light shading are used to indicate identities which occur in pairs in the same positions.

gene in lymphoid cell precursors. Dreyer and Bennett[30] have previously suggested a translocation of C genes and, more recently, this has been abandoned in favor of a detailed "copy-splice" mechanism.[31] Translocation of genes may be the basis of the phenomena of clonal expression and allelic exclusion in antibody production. Irreversible differentiation and commitment of a lymphoid precursor cell may thus occur at the time of gene translocation.

The alignment of disulfide bonds, the arrangement of symmetry axes, and the fact that proteolytic enzymes cleave the molecule to produce Fab, Fc and Fc' fragments[32] suggest that each homology region may be folded in a compact domain[29] stabilized by a single intrachain disulfide bond and linked to neighboring regions by less tightly folded stretches of polypeptide chain. Such domains would have similar but not identical tertiary structures, and each domain would

contribute to at least one active site mediating a function of that class of immunoglobulin. This domain hypothesis is consistent with the hypothesis that the molecule evolved by gene duplication as well as with the translocation hypothesis. As mentioned above, comparison of the structure and function of C_H regions in different immunoglobulin classes should reveal whether addition or deletion of homology regions and corresponding domains is a major mechanism in the evolution of these classes.

Support for the domain hypothesis would come from finding that limited proteolysis of Fab fragments yields fragments containing halves of the Fd fragments. Similar treatment of Bence-Jones proteins may produce V_L and C_L fragments.[33] Additional evidence may come from location in C_H of sites for complement fixation and skin fixation. Final proof or disproof of this hypothesis obviously rests on the results of X-ray crystallographic analysis. It is clear that a rotation axis passes through the disulfide bonds linking the heavy chains and there may be an axis of pseudosymmetry between the light and heavy chains. The locations of the interchain and intrachain disulfide bonds, the extensive homologies, and the alignment of the light and heavy chains with each other suggest that the overall relationships described above will be conserved in the three-dimensional structure regardless of the details of folding.

The exact contribution of the variable regions to the antigen combining site must also await analysis of the three-dimensional structure. It is known that the Fab fragment contains both V_L and V_H regions, and affinity-labeling experiments[24] indicate that tyrosyl residues[34, 35] in these regions are directly involved in antigen binding. The constancy of the disulfide bonds in V_H and V_L regions and their coordinate location suggest the possibility that the site is fixed by these bonds and that variations in the branches of a chain connected by each bond may be sterically arranged around the bond as a center. The closely homologous C_L and C_H1 regions may serve additionally to stabilize the structure in the face of the variation, so that both V_L and V_H can participate in the site.

In contrast to the diversity of V regions, the origin of which is still unknown,[22] the C regions appear to be quite stable in various animal species. Recent studies[8] show striking resemblances in the Fc portions of rabbit[23] and human γG immunoglobulin. In this respect, C regions, like enzymes, may have evolved to interact with specific molecules, e.g., those of the complement system. The presence of genetic differences in C regions has not so far been related to their function, but there is no reason to expect that the origin of variations in C regions will differ from other genetic polymorphisms.

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